StuDocu.com

Alignment in bioinformatics..Gene and gene regulatory networks

Biology (University of Ilorin)

StuDocu is not sponsored or endorsed by any college or university
Downloaded by bill george (monkeyabc124@outlook.com)

Lecture 2
Sequence Alignment

Burr Settles

IBS Summer Research Program 2008

bsettles@cs.wisc.edu

www.cs.wisc.edu/~bsettles/ibs08/

This document is available free of charge on StuDocu.com

Downloaded by bill george (monkeyabc124@outlook.com)

Sequence Alignment: Task Definition

- given:
  - a pair of sequences (DNA or protein)
  - a method for scoring a candidate alignment
- do:
  - determine the correspondences between substrings in the sequences such that the similarity score is maximized

Downloaded by bill george (monkeyabc124@outlook.com)

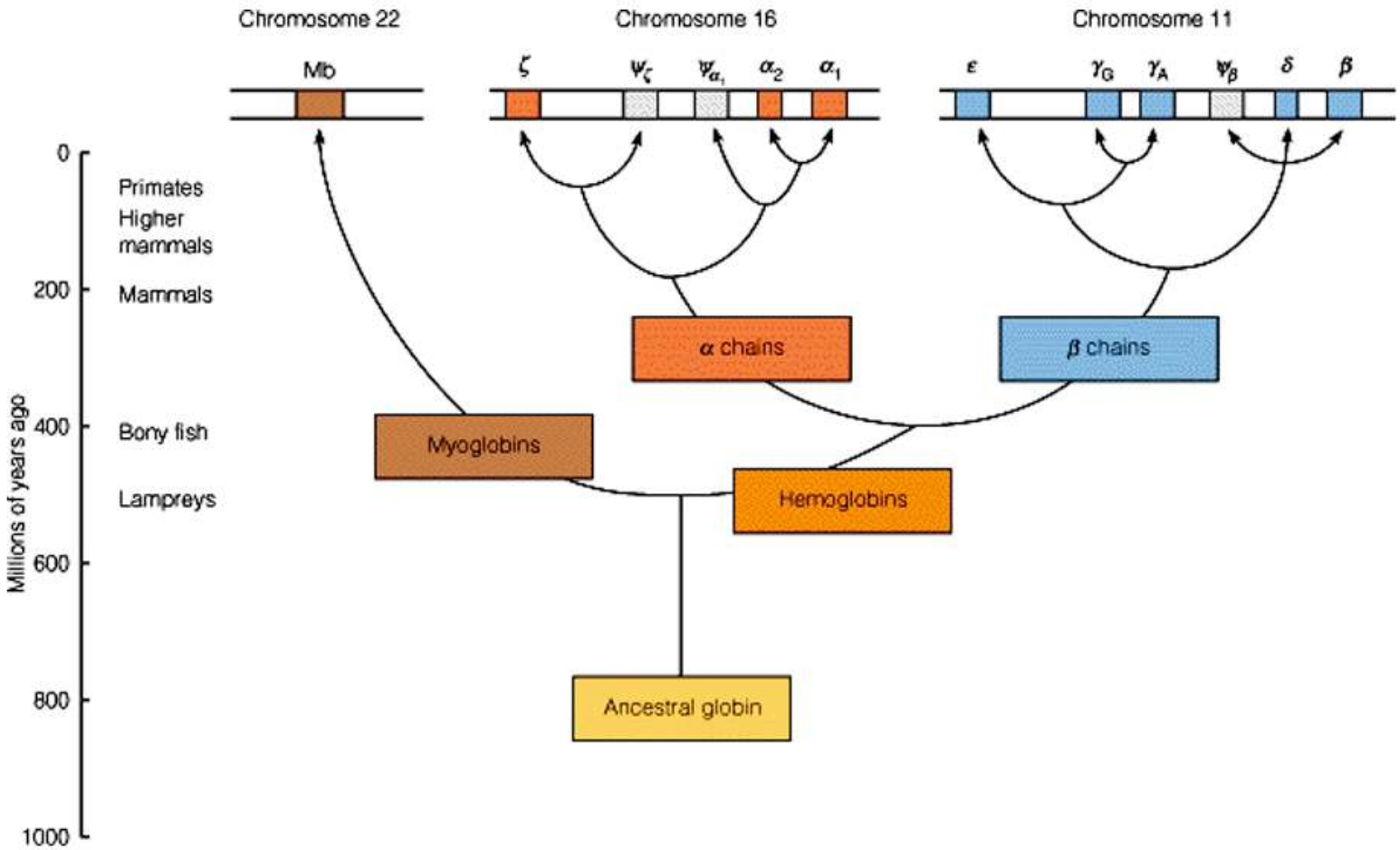
Why Do Alignment?

- *homology*: similarity due to descent from a common ancestor
- often we can infer homology from similarity
- thus we can sometimes infer structure/function from sequence similarity

This document is available free of charge on StuDocu.com

Downloaded by bill george (monkeyabc124@outlook.com)

Homology Example: Evolution of the Globins



Downloaded by bill george (monkeyabc124@outlook.com)

Homology

- homologous sequences can be divided into two groups
  - *orthologous sequences*: sequences that differ because they are found in different species (e.g. human α -globin and mouse α-globin)
  - *paralogous sequences*: sequences that differ because of a gene duplication event (e.g. human α-globin and human β-globin, various versions of both)

This document is available free of charge on StuDocu.com

Downloaded by bill george (monkeyabc124@outlook.com)

Issues in Sequence Alignment

- the sequences we're comparing probably differ in length
- there may be only a relatively small region in the sequences that match
- we want to allow partial matches (i.e. some amino acid pairs are more substitutable than others)
- variable length regions may have been inserted/deleted from the common ancestral sequence

Downloaded by bill george (monkeyabc124@outlook.com)

Sequence Variations

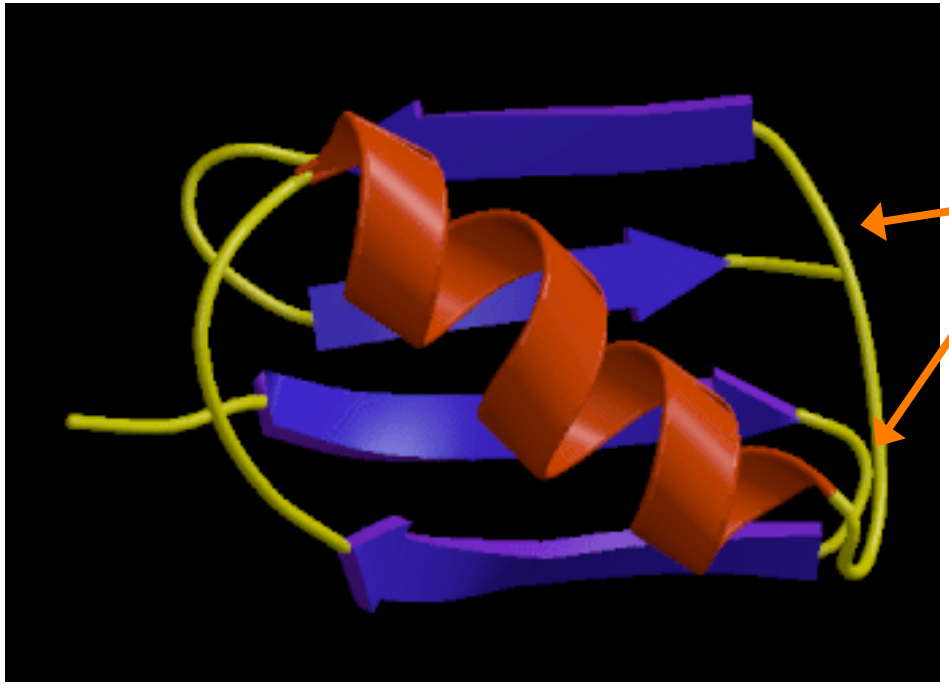
- sequences may have diverged from a common ancestor through various types of mutations:
  - substitutions (A**C**GA → A**G**GA)
  - insertions (ACGA → AC**CGGA**GA)
  - deletions (A**CGGA**GA → AGA)
- the latter two will result in *gaps* in alignments

This document is available free of charge on StuDocu.com

Downloaded by bill george (monkeyabc124@outlook.com)

Insertions, Deletions and Protein Structure

- Why is it that two "similar" sequences may have large insertions/deletions?
  - some insertions and deletions may not significantly affect the structure of a protein



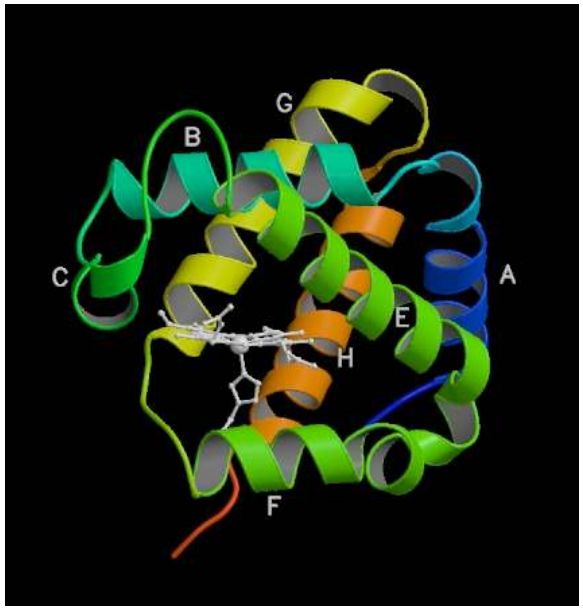
loop structures: insertions/deletions here not so significant

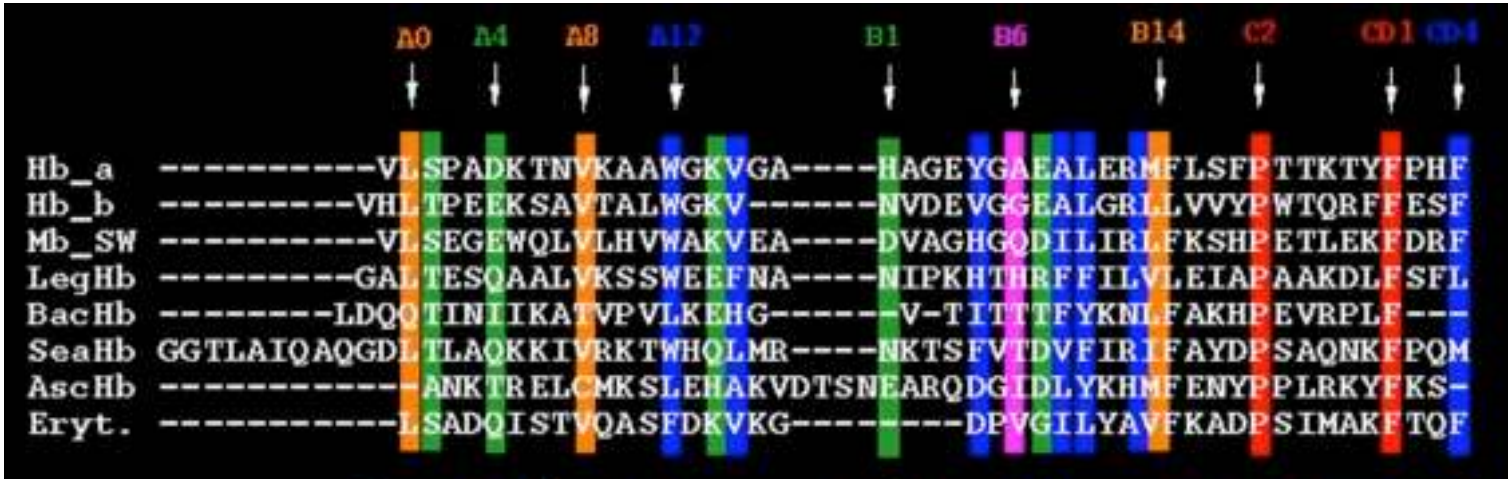
Downloaded by bill george (monkeyabc124@outlook.com)

Example Alignment: Globins

- figure at right shows prototypical structure of globins

- figure below shows part of alignment for 8 globins (-'s indicate gaps)





This document is available free of charge on StuDocu.com

Downloaded by bill george (monkeyabc124@outlook.com)

Three Key Questions

- Q1: what do we want to align?
- Q2: how do we “score” an alignment?
- Q3: how do we find the “best” alignment?

Downloaded by bill george (monkeyabc124@outlook.com)

Q1: What Do We Want to Align?

- *global alignment*: find best match of both sequences in their entirety
- *local alignment*: find best subsequence match
- *semi-global alignment*: find best match without penalizing gaps on the ends of the alignment

This document is available free of charge on StuDocu.com

Downloaded by bill george (monkeyabc124@outlook.com)

The Space of Global Alignments

- some possible global alignments for `ELV` and `VIS`

```
ELV        -ELV        --ELV        ELV-
VIS        VIS-        VIS--        -VIS


E-LV        ELV--        EL-V
VIS-        --VIS        -VIS
```

Downloaded by bill george (monkeyabc124@outlook.com)

Q2: How Do We Score Alignments?

- gap penalty function
  - $w(k)$ indicates cost of a gap of length k

- substitution matrix
  - $s(a,b)$ indicates score of aligning character a with character b

This document is available free of charge on StuDocu.com

Downloaded by bill george (monkeyabc124@outlook.com)

Linear Gap Penalty Function

- different gap penalty functions require somewhat different dynamic programming algorithms
- the simplest case is when a linear gap function is used

$$w(k) = g \times k$$

where g is a constant

- we'll start by considering this case

Downloaded by bill george (monkeyabc124@outlook.com)

Scoring an Alignment

- the score of an alignment is the sum of the scores for pairs of aligned characters plus the scores for gaps
- example: given the following alignment

```
VAHV---D--DMPNALSALSDLHAHKL
AIQLQVTGVVVTDATLKNLGSVHVSKG
```

- we would score it by

$$s(\mathrm{V,A}) + s(\mathrm{A,I}) + s(\mathrm{H,Q}) + s(\mathrm{V,L}) + 3g + s(\mathrm{D,G}) + 2g \ldots$$

This document is available free of charge on StuDocu.com

Downloaded by bill george (monkeyabc124@outlook.com)

Q3: How Do We Find the Best Alignment?

- simple approach: compute & score all possible alignments
- but there are

$$\binom{2n}{n} = \frac{(2n)!}{(n!)^2} \approx \frac{2^{2n}}{\sqrt{\pi n}}$$

possible global alignments for 2 sequences of length n

- e.g. two sequences of length 100 have $\approx 10^{77}$ possible alignments

Downloaded by bill george (monkeyabc124@outlook.com)

Pairwise Alignment Via Dynamic Programming

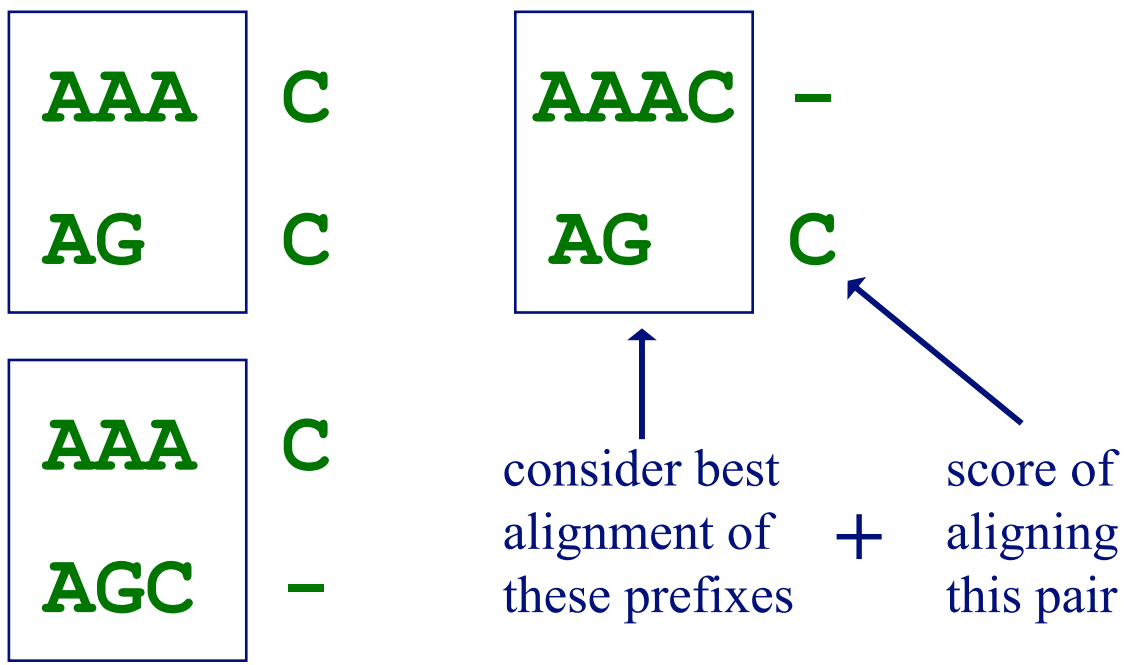
- *dynamic programming*: solve an instance of a problem by taking advantage of solutions for subparts of the problem
  - reduce problem of best alignment of two sequences to best alignment of all prefixes of the sequences
  - avoid recalculating the scores already considered
    - example: Fibonacci sequence 1, 1, 2, 3, 5, 8, 13, 21, 34…
- first used in alignment by Needleman & Wunsch, *Journal of Molecular Biology*, 1970

This document is available free of charge on StuDocu.com

Downloaded by bill george (monkeyabc124@outlook.com)

Dynamic Programming Idea

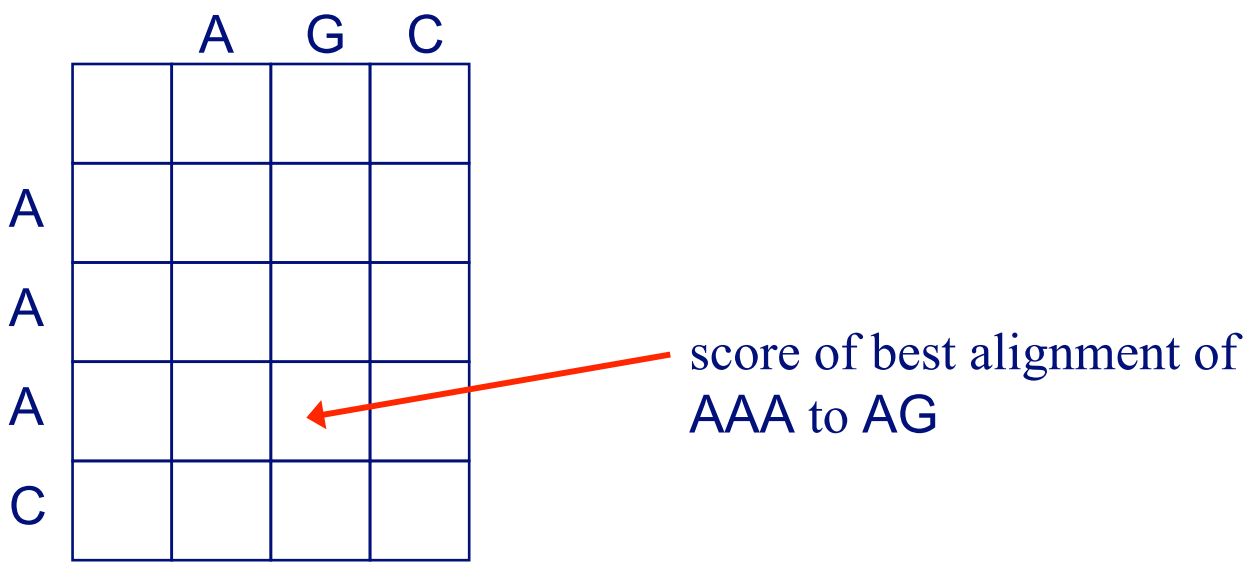
- consider last step in computing alignment of **AAAC** with **AGC**
- three possible options; in each we'll choose a different pairing for end of alignment, and add this to best alignment of previous characters



Downloaded by bill george (monkeyabc124@outlook.com)

Dynamic Programming Idea

- given an n-character sequence x, and an m-character sequence y
- construct an $(n+1) \times (m+1)$ matrix F
- $F(i, j)$ = score of the best alignment of $x[1 \ldots i]$ with $y[1 \ldots j]$



This document is available free of charge on StuDocu.com
Downloaded by bill george (monkeyabc124@outlook.com)

Needleman-Wunch Algorithm

- one way to specify the DP is in terms of its recurrence relation:

match x_i with y_j

$$F(i,j) = \max \begin{cases} F(i-1,j-1) + s(x_i, y_j) \\ F(i-1,j) + g \\ F(i,j-1) + g \end{cases}$$

insertion in x

insertion in y

Downloaded by bill george (monkeyabc124@outlook.com)

DP Algorithm Sketch: Global Alignment

- initialize first row and column of matrix
- fill in rest of matrix from top to bottom, left to right
- for each $F(i, j)$, save pointer(s) to cell(s) that resulted in best score
- $F(m, n)$ holds the optimal alignment score; trace pointers back from $F(m, n)$ to $F(0, 0)$ to recover alignment

This document is available free of charge on StuDocu.com

Downloaded by bill george (monkeyabc124@outlook.com)

Initializing Matrix

		A	G	C
	0	g	$2g$	$3g$
A	g			
A	$2g$			
A	$3g$			
C	$4g$			

Downloaded by bill george (monkeyabc124@outlook.com)

Global Alignment Example

- suppose we choose the following scoring scheme:

$$s(x_i, y_i) = \begin{cases} +1 & \text{when } x_i = y_i \\ -1 & \text{when } x_i \neq y_i \end{cases}$$

g (penalty for aligning with a gap) = -2

This document is available free of charge on StuDocu.com

Downloaded by bill george (monkeyabc124@outlook.com)

Global Alignment Example

		A	G	C
A				
A				
A				
C				

$$s(x_i, y_i) = \begin{cases} +1 & \text{when } x_i = y_i \\ -1 & \text{when } x_i \neq y_i \end{cases}$$

$$g = -2$$

Downloaded by bill george (monkeyabc124@outlook.com)

Global Alignment Example

		A	G	C
	0	-2	-4	-6
A	-2	1	-1	-3
A	-4	-1	0	-2
A	-6	-3	-2	-1
C	-8	-5	-4	-1

one optimal alignment

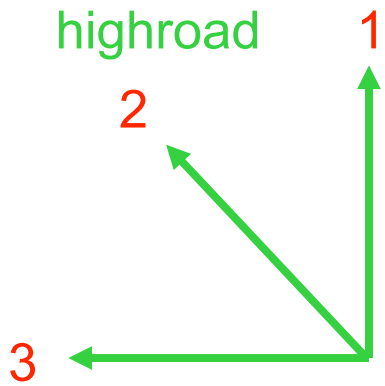
x:	A	A	A	C
y:	A	G	-	C

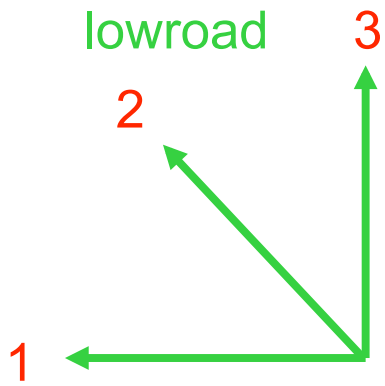
This document is available free of charge on StuDocu.com

Downloaded by bill george (monkeyabc124@outlook.com)

Equally Optimal Alignments

- many optimal alignments may exist for a given pair of sequences
- can use preference ordering over paths when doing traceback





- *highroad* and *lowroad* alignments show the two most different optimal alignments

Downloaded by bill george (monkeyabc124@outlook.com)

Highroad & Lowroad Alignments

		A	G	C
	0	-2	-4	-6
A	-2	1	-1	-3
A	-4	-1	0	-2
A	-6	-3	-2	-1
C	-8	-5	-4	-1

highroad alignment

x:	A	A	A	C
y:	A	G	-	C

lowroad alignment

x:	A	A	A	C
y:	-	A	G	C

This document is available free of charge on StuDocu.com

Downloaded by bill george (monkeyabc124@outlook.com)

DP Comments

- works for either DNA or protein sequences, although the substitution matrices used differ
- finds an optimal alignment
- the exact algorithm (and computational complexity) depends on gap penalty function (we'll come back to this)

Downloaded by bill george (monkeyabc124@outlook.com)

Local Alignment

- so far we have discussed *global alignment*, where we are looking for best match between sequences from one end to the other
- more commonly, we will want a *local alignment*, the best match between subsequences of x and y

This document is available free of charge on StuDocu.com

Downloaded by bill george (monkeyabc124@outlook.com)

Local Alignment Motivation

- useful for comparing protein sequences that share a common *motif* (conserved pattern) or *domain* (independently folded unit) but differ elsewhere
- useful for comparing DNA sequences that share a similar *motif* but differ elsewhere
- useful for comparing protein sequences against *genomic DNA sequences* (long stretches of uncharacterized sequence)
- more sensitive when comparing highly diverged sequences

Downloaded by bill george (monkeyabc124@outlook.com)

Local Alignment DP Algorithm

- original formulation: Smith & Waterman, *Journal of Molecular Biology*, 1981

- interpretation of array values is somewhat different
  - $F(i, j)$ = score of the best alignment of <u>a suffix of</u> $x[1 \ldots i]$ and <u>a suffix of</u> $y[1 \ldots j]$

This document is available free of charge on StuDocu.com

Downloaded by bill george (monkeyabc124@outlook.com)

Local Alignment DP Algorithm

- the recurrence relation is slightly different than for global algorithm

$$F(i,j) = \max \begin{cases} F(i-1, j-1) + s(x_i, y_j) \\ F(i-1, j) + g \\ F(i, j-1) + g \\ 0 \end{cases}$$

Downloaded by bill george (monkeyabc124@outlook.com)

Local Alignment DP Algorithm

- initialization: first row and first column initialized with 0's
- traceback:
  - find maximum value of $F(i, j)$; can be <u>anywhere</u> in matrix
  - stop when we get to a cell with value 0

This document is available free of charge on StuDocu.com

Downloaded by bill george (monkeyabc124@outlook.com)

Local Alignment Example

$s(x_i, y_i) =$

$+1$ when $x_i = y_i$

-1 when $x_i \neq y_i$

$g = -2$

		A	A	G	A
T					
T					
A					
A					
G					

Downloaded by bill george (monkeyabc124@outlook.com)

Local Alignment Example

x: A A G

y: A A G

		A	A	G	A
	0	0	0	0	0
T	0	0	0	0	0
T	0	0	0	0	0
A	0	1	1	0	1
A	0	1	2	0	1
G	0	0	0	3	1

This document is available free of charge on StuDocu.com

Downloaded by bill george (monkeyabc124@outlook.com)

More On Gap Penalty Functions

- a gap of length k is more probable than k gaps of length 1
  - a gap may be due to a single mutational event that inserted/deleted a stretch of characters
  - separated gaps are probably due to distinct mutational events
- a linear gap penalty function treats these cases the same
- it is more common to use an *affine* gap penalty function, which involves two terms:
  - a penalty h associated with <u>opening</u> a gap
  - a smaller penalty g for <u>extending</u> the gap

Downloaded by bill george (monkeyabc124@outlook.com)

Gap Penalty Functions

- linear

$$w(k) = gk$$

- affine

$$w(k) = \begin{cases} h + gk, & k \geq 1 \\ 0, & k = 0 \end{cases}$$

This document is available free of charge on StuDocu.com

Downloaded by bill george (monkeyabc124@outlook.com)

Dynamic Programming for the Affine Gap Penalty Case

- to do in $O(n^2)$ time, need 3 matrices instead of 1

$M(i, j)$	best score given that $x[i]$ is aligned to $y[j]$
$I_x(i, j)$	best score given that $x[i]$ is aligned to a gap
$I_y(i, j)$	best score given that $y[j]$ is aligned to a gap

Downloaded by bill george (monkeyabc124@outlook.com)

Global Alignment DP for the Affine Gap Penalty Case

$$M(i,j) = \max \begin{cases} M(i-1,j-1) + s(x_i, y_j) & \text{match } x_i \text{ with } y_j \\ I_x(i-1,j-1) + s(x_i, y_j) & \text{insertion in } x \\ I_y(i-1,j-1) + s(x_i, y_j) & \text{insertion in } y \end{cases}$$

$$I_x(i,j) = \max \begin{cases} M(i-1,j) + h + g & \text{open gap in } x \\ I_x(i-1,j) + g & \text{extend gap in } x \end{cases}$$

$$I_y(i,j) = \max \begin{cases} M(i,j-1) + h + g & \text{open gap in } y \\ I_y(i,j-1) + g & \text{extend gap in } y \end{cases}$$

This document is available free of charge on StuDocu.com

Downloaded by bill george (monkeyabc124@outlook.com)

Global Alignment DP for the Affine Gap Penalty Case

- initialization

$$M(0,0) = 0$$

$$I_x(i,0) = h + g \times i$$

$$I_y(0,j) = h + g \times j$$

other cells in top row and leftmost column = $-\infty$

- traceback
  - start at largest of $M(m,n), I_x(m,n), I_y(m,n)$
  - stop at any of $M(0,0), I_x(0,0), I_y(0,0)$
  - note that pointers may traverse all three matrices

Downloaded by bill george (monkeyabc124@outlook.com)

Global Alignment Example

(Affine Gap Penalty)

h = -3, g = -1

		A	C	A	C	T
	M : 0	-∞	-∞	-∞	-∞	-∞
	I_x : -3	-∞	-∞	-∞	-∞	-∞
	I_y : -3	-4	-5	-6	-7	-8
A	-∞	1	-5	-4	-7	-8
	-4	-∞	-∞	-∞	-∞	-∞
	-∞	-∞	-3	-4	-5	-6
A	-∞	-3	0	-2	-5	-6
	-5	-3	-9	-8	-11	-12
	-∞	-∞	-7	-4	-5	-6
T	-∞	-6	-4	-1	-3	-4
	-6	-4	-4	-6	-9	-10
	-∞	-∞	-10	-8	-5	-6

This document is available free of charge on StuDocu.com

Downloaded by bill george (monkeyabc124@outlook.com)

Global Alignment Example (Continued)

		A	C	A	C	T
	M : 0 I_x : -3 I_y : -3	-∞ -∞ -4	-∞ -∞ -5	-∞ -∞ -6	-∞ -∞ -7	-∞ -∞ -8
A	-∞ -4 -∞	1 -∞ -∞	-5 -∞ -3	-4 -∞ -4	-7 -∞ -5	-8 -∞ -6
A	-∞ -5 -∞	-3 -3 -∞	0 -9 -7	-2 -8 -4	-5 -11 -5	-6 -12 -6
T	-∞ -6 -∞	-6 -4 -∞	-4 -4 -10	-1 -6 -8	-3 -9 -5	-4 -10 -6

three optimal alignments:

```
ACACT      ACACT      ACACT
AA--T      A--AT      --AAT
```

Downloaded by bill george (monkeyabc124@outlook.com)

Local Alignment DP for the Affine Gap Penalty Case

$$M(i,j) = \max \begin{cases} M(i-1,j-1) + s(x_i, y_j) \\ I_x(i-1,j-1) + s(x_i, y_j) \\ I_y(i-1,j-1) + s(x_i, y_j) \\ 0 \end{cases}$$

$$I_x(i,j) = \max \begin{cases} M(i-1,j) + h + g \\ I_x(i-1,j) + g \end{cases}$$

$$I_y(i,j) = \max \begin{cases} M(i,j-1) + h + g \\ I_y(i,j-1) + g \end{cases}$$

This document is available free of charge on StuDocu.com

Downloaded by bill george (monkeyabc124@outlook.com)

Local Alignment DP for the Affine Gap Penalty Case

- initialization

  $M(0,0) = 0$

  $M(i,0) = 0$

  $M(0,j) = 0$

  cells in top row and leftmost column of $I_x, I_y = -\infty$

- traceback
  - start at largest $M(i,j)$
  - stop at $M(i,j) = 0$

Downloaded by bill george (monkeyabc124@outlook.com)

Gap Penalty Functions

- linear: $w(k) = gk$

- affine:

$$w(k) = \begin{cases} h + gk, & k \geq 1 \\ 0, & k = 0 \end{cases}$$

- concave: a function for which the following holds for all $k, l, m \geq 0$

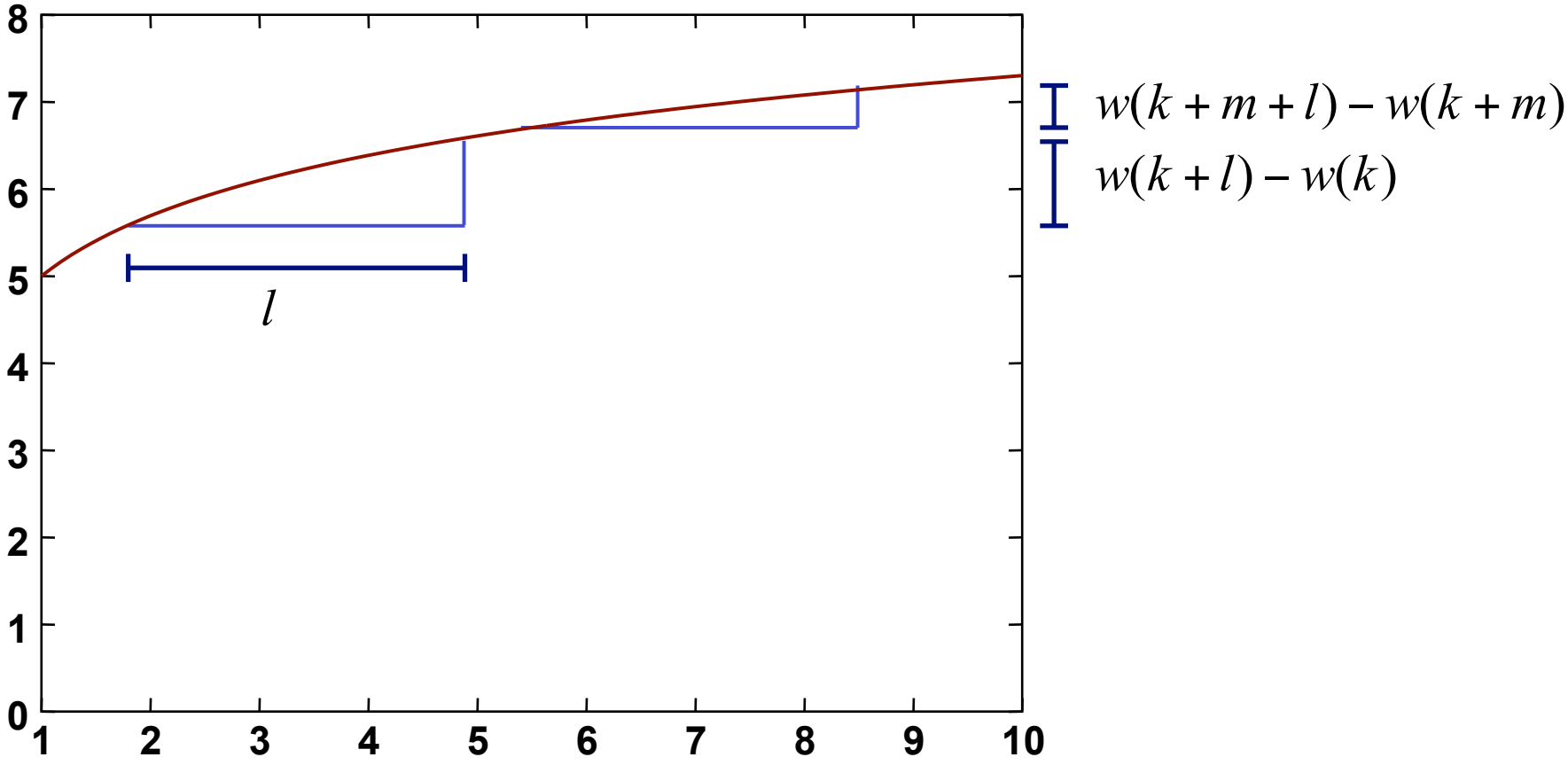
$$w(k + m + l) - w(k + m) \leq w(k + l) - w(k)$$

e.g. $w(k) = h + g \times \log(k)$

This document is available free of charge on StuDocu.com

Downloaded by bill george (monkeyabc124@outlook.com)

Concave Gap Penalty Functions



$$w(k+m+l)-w(k+m) \le w(k+l)-w(k)$$

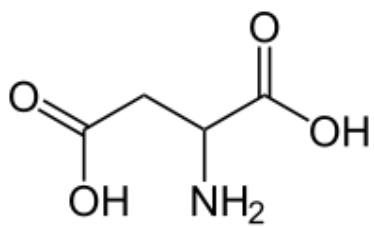
Downloaded by bill george (monkeyabc124@outlook.com)

More On Scoring Matches

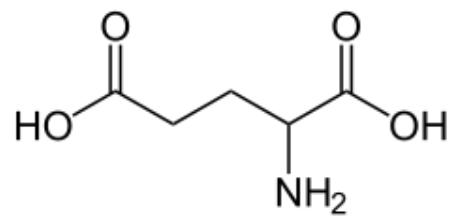
- so far, we've discussed multiple gap penalty functions, but only one match-scoring scheme:

$$s(x_i, y_i) = \begin{cases} +1 & \text{when } x_i = y_i \\ -1 & \text{when } x_i \neq y_i \end{cases}$$

- for protein sequence alignment, some amino acids have similar structures and can be substituted in nature:



aspartic acid (D)



glutamic acid (E)

This document is available free of charge on StuDocu.com

Downloaded by bill george (monkeyabc124@outlook.com)

Substitution Matrices

- two popular sets of matrices for protein sequences
  - PAM matrices [Dayhoff *et al.,* 1978]
  - BLOSUM matrices [Henikoff & Henikoff, 1992]

- both try to capture the the relative substitutability of amino acid pairs in the context of evolution

Downloaded by bill george (monkeyabc124@outlook.com)

BLOSUM62 Matrix

BLOSUM62

	A	R	N	D	C	Q	E	G	H	I	L	K	M	F	P	S	T	W	Y	V	X
A	4																				
R	-1	5																			
N	-2	0	6																		
D	-2	-2	1	6																	
C	0	-3	-3	-3	9																
Q	-1	1	0	0	-3	5															
E	-1	0	0	2	-4	2	5														
G	0	-2	0	-1	-3	-2	-2	6													
H	-2	0	1	-1	-3	0	0	-2	8												
I	-1	-3	-3	-3	-1	-3	-3	-4	-3	4											
L	-1	-2	-3	-4	-1	-2	-3	-4	-3	2	4										
K	-1	2	0	-1	-3	1	1	-2	-1	-3	-2	5									
M	-1	-1	-2	-3	-1	0	-2	-3	-2	1	2	-1	5								
F	-2	-3	-3	-3	-2	-3	-3	-3	-1	0	0	-3	0	6							
P	-1	-2	-2	-1	-3	-1	-1	-2	-2	-3	-3	-1	-2	-4	7						
S	1	-1	1	0	-1	0	0	0	-1	-2	-2	0	-1	-2	-1	4					
T	0	-1	0	-1	-1	-1	-1	-2	-2	-1	-1	-1	-1	-2	-1	1	5				
W	-3	-3	-4	-4	-2	-2	-3	-2	-2	-3	-2	-3	-1	1	-4	-3	-2	11			
Y	-2	-2	-2	-3	-2	-1	-2	-3	2	-1	-1	-2	-1	3	-3	-2	-2	2	7		
V	0	-3	-3	-3	-1	-2	-2	-3	-3	3	1	-2	1	-1	-2	-2	0	-3	-1	4	
X	0	-1	-1	-1	-2	-1	-1	-1	-1	-1	-1	-1	-1	-1	-2	0	0	-2	-1	-1	-1

Positive for chemically similar substitution

Common amino acids have low weights

Rare amino acids have high weights

This document is available free of charge on StuDocu.com

Downloaded by bill george (monkeyabc124@outlook.com)

Heuristic Methods

- the algorithms we learned today take $O(nm)$ time to align sequences, which is too slow for searching large databases
  - imagine an internet search engine, but where queries and results are protein sequences
- heuristic methods do fast approximation to dynamic programming
  - example: BLAST [Altschul *et al*., 1990; Altschul et al., 1997]
  - break sequence into small (e.g. 3 base pair) "words"
  - scan database for word matches
  - extend all matches to seek high-scoring alignments
  - tradeoff: sensitivity for speed

Downloaded by bill george (monkeyabc124@outlook.com)

Multiple Sequence Alignment

- we've only discussed aligning 2 sequences, but we may want to do more
- discover common motifs in a set of sequences (e.g. DNA sequences that bind the same protein)
- characterize a set of sequences (e.g. a protein family)
- much more complex

```
G G W W R G d y . g g k k q L W F P S N Y V
I G W L N G y n e t t g e r G D F P G T Y V
P N W W E G q l . . n n r r G I F P S N Y V
D E W W Q A r r . . d e q i G I V P S K - -
G E W W K A q s . . t g q e G F I P F N F V
G D W W L A r s . . s g q t G Y I P S N Y V
G D W W D A e l . . k g r r G K V P S N Y L
- D W W E A r s l s s g h r G Y V P S N Y V
G D W W Y A r s l i t n s e G Y I P S T Y V
G E W W K A r s l a t r k e G Y I P S N Y V
G D W W L A r s l v t g r e G Y V P S N F V
G E W W K A k s l s s k r e G F I P S N Y V
G E W C E A q t . k n g q . G W V P S N Y I
S D W W R V v n l t t r q e G L I P L N F V
L P W W R A r d . k n g q e G Y I P S N Y I
R D W W E F r s k t v y t p G Y Y E S G Y V
E H W W K V k d . a l g n v G Y I P S N Y V
I H W W R V q d . r n g h e G Y V P S S Y L
K D W W K V e v . . n d r q G F V P A A Y V
V G W M P G l n e r t r q r G D F P G T Y V
P D W W E G e l . . n g q r G V F P A S Y V
E N W W N G e i . . g n r k G I F P A T Y V
E E W L E G e c . . k g k v G I F P K V F V
G G W W K G d y . g t r i q Q Y F P S N Y V
D G W W R G s y . . n g q v G W F P S N Y V
Q G W W R G e i . . y g r v G W F P A N Y V
G R W W K A r r . a n g e t G I I P S N Y V
G G W T Q G e l . k s g q k G W A P T N Y L
G D W W E A r s n . t g e n G Y I P S N Y V
N D W W T G r t . . n g k e G I F P A N Y V
```

Figure from A. Krogh, An Introduction to Hidden Markov Models for Biological Sequences

This document is available free of charge on StuDocu.com

Downloaded by bill george (monkeyabc124@outlook.com)

Next Time…

- basic molecular biology
- sequence alignment
- **probabilistic sequence models**
- gene expression analysis
- protein structure prediction
  - by Ameet Soni

Downloaded by bill george (monkeyabc124@outlook.com)